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CS1 (CRACC, CD319) Induces Proliferation and Autocrine Cytokine Expression on Human B Lymphocytes

Jae Kyung Lee,* Stephen O. Mathew,† Swapnil V. Vaidya,§ Pappanaicken R. Kumaresan,§ and Porunelloor A. Mathew2†

CS1 (CRACC, CD319), a member of the CD2 family of cell surface receptors, is implicated in the activation of NK cell-mediated cytotoxicity. Previous studies showed that CS1 is also expressed on activated B cells. However, the functional role of CS1 in human B-lymphocytes is not known. Two isoforms of CS1, CS1-L and CS1-S, are expressed in human NK cells that differentially regulate NK cell function. CS1-L contains immunoreceptor tyrosine-based switch motifs in its cytoplasmic domain whereas CS1-S lacks immunoreceptor tyrosine-based switch motifs. In this study, we show that human B lymphocytes express only the CS1-L isoform, and its expression is up-regulated upon B cell activation with various stimulators. Moreover, anti-CS1 mAb strongly enhanced proliferation of both freshly isolated as well as activated B cells. The enhanced proliferation effects of CS1 were most prominent on B cells activated by anti-CD40 mAbs and/or hrIL-4. The effects of CS1 on B cell proliferation were shown on both naive and memory B cells. Human cytokine microarray and quantitative real-time PCR results indicated that CS1 activation enhanced mRNA transcripts of flt3 ligand, lymphotixin A, TNF, and IL-14. Neutralizing Abs against lymphotixin A, TNF-α, and/or flt3 ligand abolished the ability of CS1 on the B cell proliferation. These results suggest that activation of B lymphocytes, through surface CS1, may be mediated through secretion of autocrine cytokines and CS1 may play a role in the regulation of B lymphocyte proliferation during immune responses. *The Journal of Immunology, 2007, 179: 4672–4678.

The proliferation and differentiation of lymphocytes are regulated by receptors localized on the cell surface. Especially, activation of B cells is initiated by the recognition of specific Ag by cell surface Ig, and it is pivotal in determining the Ag specificity of the response. Cytokines derived from Th2 such as IL-4, IL-5, and IL-13 and membrane-bound costimulatory molecules expressed by activated CD4+ T cells are required for subsequent proliferation, Ig production, Ig isotype switching, and differentiation of activated B cells (1, 2). Regulation of B cell proliferation and differentiation can also be mediated by monocytes through release of cytokines such as IL-8 and IL-10 (3, 4). The B cell coreceptor, CD19, has recently been shown to regulate positive selection and maturation in B lymphopoiesis (5). The CD40 on B cells interact with CD40L (CD154) on activated CD4+ T cells to provide an essential signal for T cell-dependent B cell activation (6–9). One of the CD2 family receptors, signaling lymphocyte activation molecule (SLAM, CD150)2, could induce proliferation and Ig synthesis by activated human B lymphocytes (10). SLAM has several isoforms and among them soluble secreted form of SLAM and membrane-bound form of SLAM had B cell growth promoting effects even in the absence of other stimuli and had more potent effect in the presence of polyclonal B cell stimuli and cytokines (10).

CS1 (CRACC, CD319) is a novel member of the CD2 family (11–13) and is expressed on activated B lymphocytes, NK cells, CD8+ T lymphocytes, and mature dendritic cells (11, 12). We have previously showed that it is a self ligand, and homophilic interaction of CS1 regulates NK cell cytolytic activity (14). The cytoplasmic domain of CS1 contains two immunoreceptor tyrosine-based switch motifs (ITSM) observed in some of the CD2 family members (11, 12) that provide docking sites to recruit small SH2 domain-containing adapter proteins, including Src homology 2 domain protein 1A/SLAM-associated protein (SAP) and EWS-activated transcript-2 (EAT-2) (15). A recent study identified that CS1 recruits EAT-2 and activates the PI3K and phospholipase Cγ signaling pathways in NK cells (16). In addition, human NK cells express two splice variant forms of CS1, CS1-S that lack the ITSM in the cytoplasmic domain and CS1-L, which contain two cytoplasmic ITSM motifs. Only CS1-L was able to mediate cytotoxicity in human NK cells (17).

In this study, we showed that human B lymphocytes express only the CS1-L isoform. Contrary to the previous studies in which the expression of CS1 was shown only in activated B cells, our results demonstrate that CS1 is expressed on both naive and activated B cells. The expression levels of CS1 were up-regulated upon activation with various stimulators including anti-CD40 protein; EAT-2, EWS-activated transcript-2; hr, human recombinant; RT, reverse transcriptase; LTA, lymphotixin-α; flt3L, flt3 ligand; TNFSF13B, TNF superfamily factor 13B; NHL, non-Hodgkin’s lymphoma.

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mAb, IL-4, and anti-μ mAb. Our results also demonstrated the functions of CS1 on human B-lymphocytes. The data indicated that CS11 increased proliferation of freshly isolated as well as activated B cells. Effects of CS1 on B cell proliferation were most prominent when B cells were activated with anti-CD40 mAb and/or IL-4. Interestingly, cytokine microarray and real-time PCR data showed that CS11 increased mRNA transcripts of autocrine cytokines, which are mostly involved in B cell growth.

Materials and Methods

Reagents

Purified human recombinant (hor) IL-4, IL-2, IL-10, and IL-12 were purchased from BD Biosciences. The purified anti-CD40 mAbs and anti-μ mAb were purchased from Beckman Coulter and BD Biosciences, respectively. Anti-CS1 mAb (1 G10 mAb) was generated and purified as described earlier (14).

B cell isolation and culture conditions

B cells were obtained by density gradient centrifugation over Histopaque-1077 (Sigma-Aldrich). For depletion of T cells, NK cells, monocytes, granulocytes, dendritic cells, basophiles, platelets, and early erythroid cells from PBMC, these cells were magnetically labeled using a mixture of hapten-conjugated CD2, CD4, CD11b, CD16, CD36, and anti-IgE Abs (Miltenyi Biotec). Cells were labeled for 10 min at 6–12°C with hapten Abs mixture and FcR blocking reagent (Miltenyi Biotec). After removing the unbound Abs by washing with cold PBS plus 1% BSA, the cells were incubated with anti-hapten microbead (Miltenyi Biotec) for 15 min at 6–12°C, and then magnetically labeled cells were depleted by retaining them on a magnetic column (MACS; Miltenyi Biotec). For isolation of memory B cells, the negatively isolated B cells were incubated with CD27 microbeads for 15 min at 4–8°C. The cells were washed with cold PBS plus 1% BSA, and then the magnetically labeled cells were depleted by retaining cells on a magnetic column while the negatively selected cells were the naive CD19+ CD27- B cells.

Purified total B cells, naive, and memory B cells were cultured in round-bottom 96-well plates in 0.2 ml RPMI 1640 medium supplemented with 10% FBS at a concentration of 10^5 cells/well and incubated at 37°C in a humidified atmosphere containing 5% CO2. Cultures were performed in triplicate when proliferation of B cells was studied and in quadruplets when Ig synthesis was studied.

Reverse transcriptase (RT)-PCR

Total RNA was isolated with the RNeasy mini kit (Qiagen) according to the manufacturer’s instruction. Human common cytokine array was obtained from SuperArray Bioscience Corporation and used according to manufacturer’s instructions. In brief, 2 μg of total RNA was used for generating the biotin-16-UTP-labeled cRNA (Roche). The arrays were exposed to an Alpha Innotech FluorChem Image system for image correction. The images were then analyzed using GEArray Expression Analysis Suite (SuperArray Bioscience). The level of gene expression was determined as the average density and mean has been adjusted to value of 100 and minimum positive value was 10. Background correction was set on minimum value, which is a method in which the lowest average density spot on the array is found and the average across that spot is used as the background correction value. Normalization was performed using GAPDH with comparable results. Comparison of the two arrays was performed using a fold (ratiometric analysis (x/y)). Genes showing a fold change of ≥2.5 were selected for further evaluation.

Flow cytometry

B cells were cultured as described above and harvested and washed. Cells were incubated with anti-CS1 mAb followed by PE-conjugated mouse IgG. For double staining, FITC-conjugated CD19 mAbs were used. For staining memory B cells, CD27 mAb was used. FITC- and PE-conjugated Abs with irrelevant specificity were used as negative controls. A total of 10^6 cells with light scatter characteristics of lymphocytes of each sample were analyzed using FACScan flow cytometry (Corixa EPICS XL-MCL). The mean fluorescence intensity in the binding assay represents the average of three independent experiments with similar results.

Western blotting

Approximately 2 x 10^6 cells were incubated with or without anti-CD40 mAb (10 μg/ml) for 24 min at 37°C. Cells were then lysed with 1% Nonidet P-40, 10 mM Tris (pH 7.4), 150 mM NaCl, 100 μg/ml PMSF, and protease inhibitor mixture (Sigma-Aldrich) for 30 min on ice. The lysate was then cleared by 8–10% SDS-PAGE. Membranes were probed with IgG1 mAb and anti-actin (Santa Cruz Biotechnology). Western blots were performed according to the manufacturer’s chemiluminiscence detection system instructions (Kirkgaard & Perry Laboratories).

Proliferation assay

Purified B cells were cultured in the presence of increasing concentrations of anti-CS1 mAb. B cells were also activated with anti-μ mAb (10 μg/ml) or anti-CD40 mAb (10 μg/ml) in the presence or absence of hIL-4 (5 ng/ml), hIL-2, hIL-10 or hIL-12 (100 U/ml) and proliferation was measured by [3H]-thymidine incorporation during the last 18 h of a 4-day culture. The proliferation was also measured at [3H]-thymidine incorporation during the last 18 h of a 5-day culture. Neutralizing Abs against cytokines lymphotixin-α (LTA; 0.5 μg/ml), TNF-α (0.5 μg/ml), and F53 ligand (fHL: 1 μg/ml) were also used. Cell proliferation is expressed as mean cpm ± SD for triplicate wells.

ELISA

Quantitation of IgG in cell-free supernatants was performed by an ELISA specific for human IgG (Rockland). Ninety-six-well plates were coated with isotype-specific capture Abs at 1 μg/well in 0.1 ml of 0.5 M sodium carbonate (pH 9.6) for overnight at room temperature. The plates were washed five times with a wash solution containing 50 mM Tris (pH 8.0), 0.1 M NaCl, and 0.05% Tween 20, and incubated for 30 min at room temperature with a postcoat solution (1% BSA in 50 mM Tris (pH 8.0) and 0.15 M NaCl) to block nonspecific binding. Subsequently, cell-free supernatants and standards were added in duplicate to the plate wells (100 μl/well). Dilution of the standards was made in a blocking solution to avoid binding of serum components to the wells. All plates were incubated for 1 h at room temperature, washed five times with wash solution, and incubated for 1 h at room temperature with 100 μl/well optimal concentration of isotype-specific (anti-human IgG) Abs conjugated with HRP. After washing three times, the enzyme substrate hydrogen peroxide plus 3,3’,5,5’-tetramethylbenzidine was added for 20 min at room temperature in the dark, and the reaction was stopped with 50 μl of 2 M H2SO4. Plates were read in a microplate reader and isotype concentrations were extrapolated from a reference curve. A revelation program incorporated with the microplate reader calculated the Ig concentration.

Microarray experiments and analysis

Total cellular RNA was isolated using the RNeasy mini kit (Qiagen) according to the manufacturer’s instruction. Human common cytokine array was obtained from SuperArray Bioscience Corporation and used according to manufacturer’s instructions. In brief, 2 μg of total RNA was used for generating the biotin-16-UTP-labeled cRNA (Roche). The arrays were exposed to an Alpha Innotech FluorChem Image system for image correction. The images were then analyzed using GEArray Expression Analysis Suite (SuperArray Bioscience). The level of gene expression was determined as the average density and mean has been adjusted to value of 100 and minimum positive value was 10. Background correction was set on minimum value, which is a method in which the lowest average density spot on the array is found and the average across that spot is used as the background correction value. Normalization was performed using GAPDH with comparable results. Comparison of the two arrays was performed using a fold (ratiometric analysis (x/y)). Genes showing a fold change of ≥2.5 were selected for further evaluation.

Quantitative real-time PCR

Total RNA was reverse transcribed to cDNA using Omniscript RT (Qiagen) and random primers (New England Biolabs). The RT2 PCR primer sets from SuperArray (SuperArray Biosciences) were used for each gene, which were designed to analyze the relative expression of a specific gene based on the sequence from GenBank. Primers were LTA (PPH00337A), TNF (PPH00341A), IFN-γ (PPH00308A), IL-14 (PPH00806A), TNF superfamily factor 13B (TNFSF13B) (PPH01180A), and FLT3LG (PPH06324A). RT-PCRs using 2 μg of total RNA were performed under conditions recommended by the manufacturer. PCRs were conducted in the Cepheid SmartCycler (Cepheid) in a 25-μl reaction mixture containing 12.5 μl of RT2 Real-Time SYBR Green PCR Master Mixture (SuperArray Bioscience), 1.0 μl of RT2 PCR primer set, and 11.5 μl of RNase-free H2O containing a diluted template cDNA. Samples were preincubated for 15 min at 95°C, then subjected to 40 cycles of amplification at 95°C for 30 s for denaturing, and at 55°C for 30 s for annealing-extension. Data were displayed by SmartCycler Software 2.0 (Cepheid). The relative mRNA unit for a given gene measured from a single reverse-transcription reaction was divided by the value obtained for β-actin.

Statistical analysis

Statistical analysis was done using Student’s t test for two samples with equal variance. A p value of 0.05 or less was considered significant.
Results

Human B lymphocytes express only CS1-L isoform

The expression of CS1 on human B lymphocytes was studied by RT-PCR. In an earlier study, we found that there are two isoforms of CS1 expressed on human NK cells: CS1-L containing two ITSMs in the cytoplasmic domain and CS1-S, which does not contain ITSMs (17). Interestingly, the RT-PCR result indicates human B cells only expressed the long isoform of CS1. The stimulation of isolated B cells with anti-CD40 mAb for 72 h did not alter the pattern of mRNA expression (Fig. 1 A).

Next, we studied the surface expression of CS1 on B cells by performing double immunofluorescence using mAbs specific for CD19 and CS1. Freshly isolated human B cells were incubated in medium or with anti-CD40 mAbs for 24 h and stained with FITC-conjugated anti-CD19 mAbs and 1G10 mAbs followed by PE-conjugated secondary Abs. C, Protein expression of CS1 on human B cells. Negatively selected human B cells were incubated in medium or with anti-CD40 mAbs for 24 h. The lyse were separated by 7.5% SDS-PAGE. Membranes were probed with 1G10 mAb and anti-actin mAb.

Expression of CS1 is up-regulated upon B cell activation

We next studied the effects of various other stimulators of CS1 expression on B cells. Purified human B cells were incubated with stimulators including anti-CD40 mAb, IL-4, and anti-μ mAb. The levels of CS1 on B cells that were incubated in medium have not changed throughout 72 h. However, anti-CD40 mAbs, IL-4, and

FIGURE 1. Expression of CS1 on human B cells. A, mRNA expression of CS1 on B cells. Fresh isolated human B cells of a healthy donor were cultured in medium for 0 h, 72 h, and 72 h with the stimulation of anti-CD40 mAbs (10 μg/ml). RT-PCR was performed using CS1 primers and products were run on a 1.2% agarose gel and stained with ethidium bromide. Molecular mass standards are indicated on the left. B, Surface expression of CS1 on human B cells. Negatively selected human B cells were incubated in medium or with anti-CD40 mAbs for 24 h and stained with FITC-conjugated anti-CD19 mAbs and 1G10 mAbs followed by PE-conjugated secondary Abs. C, Protein expression of CS1 on human B cells. Negatively selected human B cells were incubated in medium or with anti-CD40 mAbs for 24 h. The lysate were separated by 7.5% SDS-PAGE. Membranes were probed with 1G10 mAb and anti-actin mAb.

FIGURE 2. Effects of anti-CD40 mAb-activated B cells on CS1 expression. Negatively selected human B cells were cultured in medium, anti-CD40 mAb (10 μg/ml), hrIL-4 (5 ng/ml), or anti-μ mAb (10 μg/ml) for the indicated time points. Thereafter, the cell were harvested, washed, and stained with mAb CD19-FITC and anti-CS1 mAb, followed by PE-conjugated secondary Ab. Open and filled histograms represent staining with control Ab and mAb 1G10, respectively. CS1 expression on CD19 positive cells was analyzed using a FACScan flow cytometer. Numbers in the histogram indicate mean fluorescence intensity values. The data shown are representative of three independent experiments.

FIGURE 3. Effect of 1G10 mAb on proliferation of freshly isolated and activated human B cells. A, Negatively selected human B cells were cultured in the presence of increasing concentration (μg/ml) of 1G10 mAb for 4 days. IL-4 (5 ng/ml) and IL-2 (100U/ml) were treated on B cells as positive controls. Proliferation was measured by MTS incorporation during last 4 h of a 4-day culture. B, Freshly isolated human B cells were cultured in medium in the presence of Ab (open) or anti-CS1 (1G10mAb) (filled) or anti-CD40 mAb (10 μg/ml) in the absence (open) or presence (filled) of 1G10 mAb (20 μg/ml) as indicated for 4 days. hrIL-2 (100 U/ml), IL-4 (5 ng/ml), IL-10, or IL-12 (100 U/ml) were also used to stimulate B cells. Proliferation was measured by [³H]-thymidine incorporation assay during last 18 h of a 5-day culture. Cell proliferation is expressed as mean cpm ± SD for triplicate wells from separate experiments. *, p < 0.05.
B cells was analyzed using a Coulter FC500 flow cytometer. Incubation with control Ab and CD27 mAb, respectively. CD27 expression on the cells were harvested, washed, and stained with CD27 mAb followed by anti-CD40 mAbs and IL-4 (Fig. 3A). Proliferation was measured by [3H]-thymidine incorporation assay during stimulation had the most synergistic effect on B cell proliferation. Combination with IL-4 cytokines (Fig. 3B) induced by anti-CD40 mAbs and IL-4 (Fig. 3A). Optimal effects by anti-CD40 mAbs and IL-4 (Fig. 4A). However, the CS1-stimulated proliferating B cells did not seem to show any change in the memory phenotype as there was no change in the expression of CD27+ B cells after CS1 stimulation for 5 days (Fig. 4B).

1G10 mAb induces proliferation of both naive and memory B cells

The effect of 1G10 mAb was studied on naive and memory B cells. CS1 induced greater proliferation of memory B cells as compared with naive B cells. This effect was augmented when incubated with anti-CD40 mAbs and IL-4 (Fig. 4A). However, the CS1-stimulated proliferating B cells did not seem to show any change in the memory phenotype as there was no change in the expression of CD27+ B cells after CS1 stimulation for 5 days (Fig. 4B).

CS1 stimulation did not induce Ig production

We next studied the effects of CS1 on Ig-synthesis using purified B cells cultured in the presence or absence of 1G10 mAb. Anti-CD40 mAb was used as a positive stimulator. Neither 1G10 mAb alone nor combined with anti-CD40 mAb failed to induce IgG synthesis by purified B cells, although the effect of anti-CD40 mAb in IgG production was quite obvious as a positive control (Fig. 5). This suggests that CS1 may not be involved in regulating IgG production. We also tested production of IgM and IgE, and found no induction by 1G10 mAb (data not shown).

Expressions of common cytokine genes in activated human B-lymphocytes upon CS1 stimulation

To determine the mechanism of CS1 induction of B cell proliferation, we performed cytokine microarray for the transcriptional

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levels of autocrine cytokines upon CS1 stimulation. The human common cytokine microarray contained 114 known cytokine genes. Purified human B cells were stimulated with anti-CD40 mAb and IL-4 for 4 days in the presence or absence of 1G10 mAb. Total RNAs were isolated from each sample and used for generating biotinylated cRNA probe. The results showed that 19 cytokines increased mRNA expression of cytokines on activated B cells. A Human cytokine microarray. Negatively isolated human B cells were cultured in CD40 mAb (10 μg/ml) and IL-4 (5 ng/ml) in the presence (right panel) or absence (left panel) of 1G10 mAbs (20 μg/ml) for 4 days. Total RNAs were isolated and biotinylated cRNAs were used as probes and hybridization was performed as described in Materials and Methods. The arrays were exposed to an Alpha Innotech FluorChem Image system for image correction. Normalization was performed using GAPDH with comparable results. Comparison of the two arrays was performed using a fold (ratiometric analysis (v/y)) analysis. Genes showing a fold change of >2.4 were selected for further evaluation. B Real-time quantitative PCR showing high expression of LTA, TNF, IL-14, and flt3L genes in CS1-treated CD40/IL-4-activated human B cells. Freshly isolated human B cells were incubated with CD40 (10 μg/ml) and IL-4 (5 ng/ml) in the presence (filled) or absence (open) of 1G10 mAb for 4 days. Real-time PCR of each transcript was performed. Results are presented as relative units of each transcript compared with β-actin. *, p < 0.05; **, p < 0.01; ***, p < 0.001 (compared with CS1-treated B cells).

FIGURE 6. 1G10 mAb increased mRNA expression of cytokines on activated B cells. A Human cytokine microarray. Negatively isolated human B cells were cultured in CD40 mAb (10 μg/ml) and IL-4 (5 ng/ml) in the presence (right panel) or absence (left panel) of 1G10 mAbs (20 μg/ml) for 4 days. Total RNAs were isolated and biotinylated cRNAs were used as probes and hybridization was performed as described in Materials and Methods. The arrays were exposed to an Alpha Innotech FluorChem Image system for image correction. Normalization was performed using GAPDH with comparable results. Comparison of the two arrays was performed using a fold (ratiometric analysis (v/y)) analysis. Genes showing a fold change of >2.4 were selected for further evaluation. B. Real-time quantitative PCR showing high expression of LTA, TNF, IL-14, and flt3L genes in CS1-treated CD40/IL-4-activated human B cells. Freshly isolated human B cells were incubated with CD40 (10 μg/ml) and IL-4 (5 ng/ml) in the presence (filled) or absence (open) of 1G10 mAb for 4 days. Real-time PCR of each transcript was performed. Results are presented as relative units of each transcript compared with β-actin. *, p < 0.05; **, p < 0.01; ***, p < 0.001 (compared with CS1-treated B cells).

FIGURE 7. Effect of neutralizing Abs against cytokines abolishes CS1-induced B cell proliferation. Negatively selected human B cells were cultured in medium or anti-CD40 mAb (10 μg/ml) in the presence (filled) or absence (open) of 1G10 mAb (20 μg/ml) for 4 days. Neutralizing Abs against LTA (0.5 μg/ml), TNF-α (0.5 μg/ml), and flt3L (1 μg/ml) alone and combination (comb) of all three were also used. Proliferation was measured by [3H]-thymidine incorporation assay during the last 18 h of a 5 day culture. Cell proliferation is expressed as mean cpm ± SD for triplicate wells from three separate experiments. *, p < 0.05.

Discussion
In this study, we have shown that only one isoform of CS1 (CS1-L) is expressed on human B lymphocytes and ligation of surface CS1 by the mAb 1G10-induced proliferation as well as expression of autocrine genes in B cells. B cell activation requires two signals; the first signal is delivered through its Ag receptor. The second signal is delivered by a helper T cell by the interaction between CD40L on the T cell and CD40 on the B cell. CD40 signaling activates different mediators and pathways that are related to cell growth and differentiation (18). These signaling pathways lead to the activation of transcription factors (19, 20). Through these signaling pathways, CD40 signals can induce proliferation in B cells (20–25), induction of isotype switching (26, 27), differentiation to Ab-secreting plasma cells (28, 29), and generation of memory (30), and stimulate the up-regulation of cell surface proteins such as LFA-1 (31), ICAM-1 (31, 32), CD23 (33), and B7 (34). This, along with the fact that CS1 is homophilic, led us to believe that CS1 might be one of the cell adhesion molecules regulating B cell proliferation and activation.

There are many studies that indicate a role for members of the CD2 family of cell surface molecules in activation of lymphocytes. Meuer et al. (35) showed that CD2 engagement induces T cell proliferation in the absence of TCR signaling (36), and its natural ligand, CD58 (37), on APCs reduce the concentration of Ag required for T cell activation (38). Anti-CD2 mAb also could induce proliferation of CD2 positive human B progenitor cell lines (39, 40). Moreover, blocking of CD2 on the surface of T cells inhibits the capacity of these cells to mediate productive T-B cell interactions (6). Recombinant soluble secreted form of SLAM and murine L cells transfected with cDNA encoding membrane-bound form of
SLAM induced proliferation and IgM, IgG, and IgA production by unfraccionated B cells. However, only IgM production by sorted sIgD+ B cells was observed, indicating that SLAM acts as a B cell growth and differentiation-promoting molecule, but not as an Ig isotype switch factor (10).

Our data showed that 1G10 mAb alone increased naive and memory B cell proliferation and also enhanced proliferation of activated B cells. At present, we do not know the mechanism by which CS1 induces B cell proliferation. One could envisage two probable pathways for CS1 induction of B cell proliferation: first, CS1 signaling itself could induce B cell proliferation by activating growth-related signaling pathways; or second, CS1 may induce cytokine productions to promote proliferation.

CS1 contains ITSMs in its cytoplasmic domain and ITSMs can recruit small adaptor molecules (41). The SAP family adaptors bind to the ITSMs in the cytoplasmic tail of SLAM (CD150) (42), 2B4 (CD244) (43–46), Ly-9 (CD229), and CD84 (47) via its SH2 domain. There are three members of SAP family adaptors, which include SAP, EAT-2, and EAT-2-related transducer (48). In the previous study, EAT-2 was expressed in highly purified cell-sorted B lymphocytes and macrophages in mice (49) and it has been shown that EAT-2 binds to CD84, CD150, CD244, and CD229 through its SH2 domain (50). However, recent studies showed that neither SAP nor EAT-2 is expressed in human B-lymphocytes (16, 51). CS1 could recruit EAT-2 in human NK cells (16) and SAP has been shown to bind to CS1 when the receptor was not activated (17). Although distinct signals through the different adaptors that couple the CS1 receptor are currently poorly understood, it is interesting that human B lymphocytes do not possess any of these adaptor molecules. This may implicate that the signaling pathway for CS1 receptor in B lymphocytes may be distinct from the signaling pathways in NK cells.

Second mechanism may involve CS1 induced secretion of autocrine cytokines, which are associated with B cell growth. Recent studies show that B cells may regulate immune responses by secreting B cell effector cytokines to immune modulation (52, 53). However, little is known about the factors regulating such cytokine production. Our results suggested that 1G10 mAb can enhance autocrine cytokine production by increasing cytokine mRNA expressions on CD40/IL-4-activated B cells. The mRNA of six cytokines including flt3 (fms-like tyrosine kinase 3) ligand (flt3L), IFN-γ, IL-14, LTA, TNF, and TNF superfamily factor 13B (TNFSF13B) were selected by the microarray. Real-time quantitative PCR confirmed mRNA level of LTA was increased ~3-fold, and the transcripts of flt3L, IL-14, and TNF also showed significant increase in the CS1 stimulated B cells.

Duddy et al. (54) reported that TNF-α, lymphotixin-α, and IL-6 were secreted from B cells that are stimulated by sequential BCR and CD40 stimulation. These cytokines could act as autocrine growth and differentiation factors as well as modulators of immune responses. In the presence of IL-4, engagement of CD40/CD154 or anti-CD40 mAb stimulation not only results in B cell proliferation, but also increased production of the cytokine LTA (55). In their study, LTA, also known as TNF-α, can induce proliferation of B cells and enhance CD40/IL-4-activated B cells. IL-14, called high m.w.-B cell growth factors, had been identified in non-Hodgkin’s lymphoma (NHL)-B. There was a constitutive expression of IL-14 by aggressive immunohemalomas of the B cell type NHL-B from the patient. Also, the proliferation of lymphoma B cells was increased by IL-14, suggesting that autocrine and paracrine production of IL-14 may play a significant role in the rapid proliferation of aggressive NHL-B (56). The human TNF-α gene is one of the earliest genes transcribed after stimulation of T and B cells through their Ag receptors. It has been shown that anti-CD40 mAb/IL-4 induced rapid TNF-α gene transcription. B cell proliferation induced by CD40/IL-4 stimulation was inhibited by anti-TNF-α Ab which suggests that TNF-α is a required autocrine B cell growth factor (57).

CS1 also increased flt3L mRNA expression significantly. During B cell development, interaction between B cell progenitors and stromal cells are quite important. flt3L is a potent hemopoietic cytokine that affects the growth and differentiation of progenitor and stem cells both in vivo and in vitro (58). It has been shown that flt3L synergies with IL-7 in the proliferation of committed B220+ pro-B cells and may contribute to the maintenance of an earlier pro-B cell population, implicating an important role for flt3L in the differentiation and proliferation of early B cell progenitors in vitro (59). A recent study using flt3L and IL-7 receptor-deficient mice showed that signaling through the cytokine tyrosine kinase receptor flt3 and IL-7Rα are indispensable for fetal and adult B cell development (60).

It is very interesting that all cytokines enhanced by 1G10 mAb stimulation are related to growth-promoting activity. Thus, CS1 may accelerate proliferation of CD40/IL-4 activated human B cells by inducing prolonged secretions of autocrine cytokines.

Disclosures
The authors have no financial conflict of interest.

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